Application of *Trichoderma asperellum* T34 on Maize (*Zea mays*) Seeds Protects Against Drought Stress

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Main conclusion

Coating maize seeds with the microbial plant protection product *Trichoderma asperellum* strain T34 is an effective form of inoculation that enhances plant performance when faced with drought stress, and it improves nutrient and kernel parameters differently in drought and non-stressed conditions.

Abstract

Drought is currently one of the biggest threats to maize production. *Trichoderma* spp. is mainly used in agriculture as plant protection product with secondary beneficial effects on plants: improved growth, nutrient uptake and plant immunity. Here, we studied the physiological performance of maize plants under two different water regimes (fully irrigated and drought conditions) and three different seed treatments: application of *Trichoderma asperellum* strain T34, application of a chemical fungicide (CELEST XL) or the combination of both. Regardless of water regime, T34 treatment improved kernel P and C, kernel number and dry weight. Higher populations of T34 on the rhizosphere (T34 treatment) alleviated water stress better than lower T34 populations (T34+Q treatment). Under drought, T34 treatment improved leaf relative water content, water use efficiency, *PSII* maximum efficiency and photosynthesis. T34-treated maize seeds maintained sufficient T34 populations to alleviate drought throughout crop development suggesting an optimal dose of 10^4 and 10^5 colony forming units·g^{-1} dry weight of rhizosphere under the studied conditions. This work helps to demonstrate the beneficial interaction between *T. asperellum* strain T34 and maize plants under drought.

KEYWORDS: drought stress, elemental nutrient concentration, photosynthesis, gas exchange, kernel parameters, and leaf relative water content.

Introduction

Seed treatments are a suitable way to protect seeds against environmental adversities and ensure plant stand and seedling health. They mainly consist in the external application of a given compound (or more than one), which stays attached to the seeds (Zeun et al. 2013). Currently, most seed treatments are chemical formulations (Krzyzinska et al. 2005; Zeun et al. 2013; Moya-Elizondo and Jacobsen 2016), the goal of which is to protect the seed against seed- and soil-borne diseases, and they are extensively used in all agricultural areas and with almost all crops (Zeun et al. 2013). However, agricultural practices are changing, and more natural and sustainable seed treatments are required. Slowly but steadily, seed treatments have

been evolving to include the application of active biological substances, including *Azospirillium*, *Pseudomonas*, *Azotobacter*, *Bacillus spp.* and *Trichoderma spp.*, together with various adhesive agents (Taylor et al. 1991; Gholami et al. 2009; Hu et al. 2011; Moya-Elizondo and Jacobsen 2016). Microbial inoculants have several advantages over chemical compounds: greater safety, potentially reduced environmental and human damage, targeted activity, effectiveness in small amounts, and self-multiplication controlled by the plant and the indigenous microbial populations (Singh et al. 2011).

Maize (*Zea mays*) is grown on over 170 million ha worldwide, of which 130 million ha are in less-developed countries (FAO, 2014). Low yields of these crops in sub-Saharan Africa are largely associated with drought stress and low soil fertility. Water stress decreases plant growth and development leading to a reduction in crop yield (Farooq et al. 2009). Plant tolerance to this abiotic stress is the result of extremely complex mechanisms involving molecular, biochemical and physiological processes (Medina et al. 2016). The degree of the reduction in crop yield due to this stress is dependent not only on the genotype but also on the severity and duration of the drought, the phase of plant growth and development, and environmental interactions with other factors (Medina et al. 2016).

Many studies of abiotic stress alleviation have used mycorrhizae, nitrogen-fixing bacteria (Ortiz et al. 2015), and increasingly, *Trichoderma* spp. in different crops (Pandey et al. 2016; Alwhibi et al. 2017; Fu et al. 2017). Some *Trichoderma* spp. are known to protect plants against pathogens through competing for nutrients, parasitism, antibiosis, enzyme activity and stimulating the innate immunity of plants; but they also promote growth (Howell 2003; Segarra et al. 2007; Shoresh et al. 2010; Lorito et al. 2010). Products based on *Trichoderma* spp. are commercially available as plant protection products (PPPs) for a wide range of crops (horticultural, ornamental and agricultural crops, as well as fruit and vegetables during post-harvest storage (Harman et al. 2004; Verma et al. 2007). In particular, *Trichoderma asperellum* strain T34 (T34), has been widely studied under non-stressed conditions (de Santiago et al. 2011, 2013; García-López et al. 2015) and under biotic stress (Cotxarrera et al. 2002; Trillas et al. 2006; Segarra et al. 2010; Borrero et al. 2012; Segarra et al. 2013; Fernández et al. 2014).

Here we aim to determine the effect of T34 on maize growth, kernel development, and both physiological and nutritional status under two irrigation conditions (well-watered and water-stressed conditions) via the application of three types of seed treatments: biological, chemical, and their combination. Our work focuses

77 on a rapidly growing hybrid that has some tolerance to drought and which is widely used commercially in
78 the Mediterranean region.

79 **Materials and methods**

80 **Experimental setup**

81 The experiment was performed in a glasshouse at the facilities of *Camps Experiementals, Universitat de*
82 *Barcelona* (41º23'6.259''N; 2º7'12.434''E, 60 m above sea level) with maize (*Zea mays*) seeds treated or
83 not with T34, over four months from June to October. The maize genotype was the hybrid SF9C031 from
84 *Semillas Fitó* (Barcelona, Spain). T34 was coated on the seeds at a concentration of 10^{10} cfu·g^{-1} and applied
85 at 4 kg·Tn^{-1} of seed; the chemical fungicide was applied at 10 L·Tn^{-1} of seed. Temperature and relative
86 humidity were measured throughout the experiment with a datalogger (PCE-HT 71N, PCE Ibérica,
87 Albacete, Spain) located in the center of the trial at canopy level, protected from direct light. During the
88 113 days after sowing (DAS), the average temperature was 25.3ºC (maximum 38.0ºC and minimum 14.5ºC)
89 and the relative humidity was 63%; (maximum 92.5% and minimum 27.0%). The plants were covered with
90 a shading screen extended in the hottest hours of the day, 12:00-16:00 h (UTC+2: CEST), and when
91 necessary a cooling system was activated to maintain a suitable temperature.

92 Three seed pools were used: seeds treated with the commercial biological fungicide (T34), active ingredient
93 *T. asperellum* strain T34 (Biocontrol Technologies, S.L., Barcelona, Spain); seeds treated with a
94 commercial chemical fungicide (Q), CELEST XL active ingredient Fludioxonil 25% and Metalaxyl-M 1%
95 (Syngenta Crop Protection, Basel, Switzerland); and a combination of both products (T34+Q). Previous to
96 seed treatment, all seeds were surface disinfected before any application with 2% sodium hypochlorite
97 (commercial bleach) for 10 min, and then rinsed three times with sterile distilled water before air drying in
98 a laminar flow hood at room temperature for 2 h. Maize seeds (one per pot) were sowed 10 cm from the
99 top of the substrate, using 10 L pots with coconut fiber:perlite:vermiculite (2:1:1, v/v) as an inert substrate
100 with a pH of 6.5 (25ºC) and an electrical conductivity of 1 mS·cm^{-1}. The plants were watered with
101 Hoagland's solution diluted 1:2. There were two irrigation conditions: fully irrigated and drought condition,
102 with 20 pots per treatment, giving a total of 120 pots. The pots were placed in two blocks in order to fit two
103 independent lines of drip irrigation. The pots within each block were placed randomly. The chemical
104 fungicide was applied in such a way as to mimic commercial practices with a well-used broad-spectrum
105 fungicide.

The irrigation was the same in all pots for 35 DAS (from VE to V8 maize scale (Hanway and Ritchie 1986)) using two drips per pot, which provide approximately 160±8.3 ml of water per application three times a day. The fertirrigation was applied automatically in the early morning, at the middle of the day, and in the late afternoon. Then, half watering was applied by removing one of the drips in half of the pots (mild water-stress) for a 28-day period (35-63 DAS, from V8 to R1 (silking) maize stage (Hanway and Ritchie 1986)). In this period, fertirrigation was applied three times per day, at a dose of 146±5.2 $mL \cdot pot^{-1} \cdot application^{-1}$, except for the stressed plants, where half of this watering was applied. After that, the drought stress was intensified by removing the remaining drip from the drought treatment pots (intensified water-stress) from 63 to 113 DAS (from R1 to R5 maize scale (Hanway and Ritchie 1986)). The well-watered plants (WW) continued receiving the fertirrigation three times per day with two drips per pot at a dose of 355±7.6 $mL \cdot pot^{-1} \cdot application^{-1}$. Nevertheless, both WW and water-stressed (WS) plants received additional water supply (one $application \cdot day^{-1}$, during five days distributed uniformly in this last 50-day period) to avoid complete plant desiccation in WS. The drought stage lasted 78 days and the study ended at 113 DAS. A soil moisture meter (Jellas Soil pH Meter, Jellas Technology Limited, Hong Kong) was regularly used to monitor the soil moisture in the pots. Normal soil moisture levels (4 to 7 on a scale of 0-10) were always maintained for WW, while in WS values reached "dry" (<3) during the measurements and sampling of material.

T34 population

To assess the establishment and maintenance of the fungus, its population was determined by the serial dilution method and plate counting in *Trichoderma* spp. selective medium (Chung and Hoitink. 1990). Populations were evaluated in seeds at sowing time (3 to 5 seeds per treatment, T34, Q and T34+Q) as well as at the end of the experiment in the plant rhizosphere for five pots per treatment and condition and repeated twice. Dilutions were initiated with 1 g of seeds in a tube containing 9 mL of autoclaved saline solution (NaCl 9 $g \cdot L^{-1}$), or with 10 g of substrate from the soil rhizosphere (without removing the roots) in 90 mL of autoclaved saline solution and agitated for 30 minutes in a rotatory shaker.

Scanning electron microscopy images

To check for the effective adhesion of T34 on the seed surface, seed samples were fixed in 2% paraformaldehyde and 2.5% glutaraldehyde (pH 7.4) at 4°C, then washed in phosphate buffer 5 times at 4°C and further processed with a solution containing 1% osmium tetroxide and 0.8% potassium ferrocyanide in phosphate buffer for 2 hours at 4°C and later washed with distilled water. Afterwards, the

samples were dehydrated with ethanol and dried using the critical point procedure (Polaron Critical Point Drier). The samples were mounted in a standard Scanning Electron Microscope (SEM) support and coated with Au (Fisons Instruments SEM coating system). The secondary electron images were obtained using a SEM JEOL 7001 FEG at the Electron Microscopy Unit.

Gas exchange and chlorophyll fluorescence measurements

Gas exchange parameters were determined to provide key information about plant physiology and photosynthetic machinery. Maize plants were measured with a Li-Cor 6400 system (Li-Cor, USA) using the youngest fully developed leaves. The measurements were recorded from an intermediate leaf position on one side of the central nerve. Four to five plants were used per treatment and water condition at three different stages: 63 DAS, at the end of the mild water stress (R1, silking), and at two points during intensified water stress, at 85 DAS (R3, milk stage) and, 104 DAS (R4, dough stage). The measurements were photosynthesis rate (An), transpiration (E), stomatal conductance (g_s), ratio of intercellular/extracellular CO_2 concentration (C_i/C_a), maximum quantum efficiency of photosystem II (Fv'/Fm'), and water use efficiency (WUE), calculated as the ratio An/E. These measurements were taken at saturating 1,500 $\mu mol \cdot m^{-2} \cdot s^{-1}$ irradiance and 25ºC using a 2 cm^2 leaf chamber, and performed on clear days between 3 and 9 h after dawn, when photosynthesis was most likely to peak.

Water status, yield parameters and mineral nutrients

A simple and classic method for evaluation of plant hydric status is the leaf relative water content (RWC). It was measured on the same leaves as for the gas exchange measurements. RWC was calculated using the formula: RWC (%) = ((FW-DW) / (TW-DW)) x 100; where FW is the leaf fresh weight, TW is the leaf turgid weight after 24 h imbibition in water at 4ºC, and DW is the leaf dry weight after drying the sample to constant weight in an oven at 60ºC (Barrs & Weatherley, 1962).

Since drought and T34 application can affect nutrient absorption, mineral nutrients were determined in leaves at two different growth stages (85 and 104 DAS; R3 and R4, respectively) and in kernels at harvest (113 DAS) in the *Centres Científics i Tecnològics de la Universitat de Barcelona* (CCITUB, Spain). Moreover, at the end of the experiment (113 DAS) the dry weight/number of kernels per plant, plant height and shoot fresh/dry weight were determined for each treatment and condition combination. Leaf samples were taken from the dried leaves used for the gas exchange, chlorophyll fluorescence and RWC

measurements. All the samples were finely powdered using a Mixer Mill MM400 (Retsch GmbH, Germany). Then 100 mg per sample was digested with 2 mL concentrated HNO_3 and 2 mL H_2O_2 in a Teflon container at 90°C for 3 days. Analysis of Ca, K, Mg, Fe, P, Na and S was performed by inductively coupled plasma optical emission spectrometry (ICP-OES) using an Optima-3200RL (Perkin Elmer). Analysis of B, Cu, Zn and Mn was performed by ICP mass spectrometry (ICP-MS) using an Elan 6000 (Perkin Elmer).

Carbon and nitrogen content and isotope composition

The carbon and nitrogen concentration and the stable isotope composition (‰) of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined in the leaves and kernels using an elemental analyzer (EA1108, Series 1, Carlo Erba Instruments) coupled to an isotope ratio mass spectrometer (IRMS, Delta C, Finnigan MAT) as described in Medina et al. (2016). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were expressed in δ notation for carbon and nitrogen as follows: δ (‰) = ((Sample Isotope Ratio / Standard Isotope Ratio) –1) x 1000; where the standard refers to the international secondary standards of known stable carbon and nitrogen ratios (Medina et al. 2016).

Statistical analysis

Treatment main effects and interactions were determined by one- or two-way analysis of variance (ANOVA) followed by Fisher's least significance difference (LSD) test ($P < 0.05$). Results of T34 populations, nutrient elements and isotope parameters are mean values of three replicates per treatment and condition. Nutrient and isotope determination in kernel was performed on three ears of different plants per treatment and condition, being each of them a pooled sample of all grains in each ear. Results of gas exchange and water parameters are mean values of three to five replicates per treatment and condition. Results of kernel dry weight and number are mean values of four to seven replicates per treatment and condition. Results of plant height and plant shoot weight are mean values of seven and three replicates per treatment and condition, respectively. Normality and homoscedasticity of the data was assumed in order to perform the ANOVA. Moreover, each plant represented an independent sample. The analysis was performed with GenStat 7th Edition software (VSN International Ltd., United Kingdom), while the graphics were produced with Sigma-Plot 11 software (Systat Software Inc., USA).

Results

Seed treatment and T34 population

SEM images (Fig. 1) showed the conidia of fungus attached to the seed in a stable and uniform manner. The adhesive applied surrounded the conidia and adhered it to the seed (Fig. 1a, b) while developing hyphae and conidia can be seen in the seeds once germinated (Fig. 1c, d).

Fungal population per seed (Table 1) just before sowing showed equal populations in both inoculated treatments (T34 and T34+Q) as expected. The Q treatment, on the other hand, presented negligible populations of T34, probably due to accidental contributions, but insignificantly in comparison to the treated seeds. In the rhizosphere, T34 populations were higher in T34 seed treatment, followed by seeds with T34+Q, and then Q. Interestingly, the application of the chemical reduced the proliferation of T34 by a factor of 10. Apart from that, significant results regarding water regime and treatment unexpectedly differentiated T34 populations, but to a lesser extent than for treatment only.

Physiological plant measurements

Gas exchange and chlorophyll fluorescence measurements were firstly recorded at a very early stage (35 DAS) before the application of the different water regimes (Suppl. Table S1). At this stage, these parameters were not significantly modified by the treatment factor. At 63 DAS, An and E increased significantly in the T34 treatments in comparison to Q treatment, especially under water stress conditions in T34 (Fig. 2). C_i/C_a ratio and leaf RWC were slightly reduced by WS compared to WW and C_i/C_a ratio and g_s increased in T34 compared to T34+Q and Q (Fig. 3, Suppl. Table S1). At a more advanced reproductive stage (85 DAS), An, E, g_s and Fv'/Fm' decreased under WS compared to WW (Fig. 2, Suppl. Table S1). The highest E value was reached in the treatment T34+Q under WS conditions. C_i/C_a ratio decreased under WS, with the highest value obtained in T34 under WW, although it was significantly decreased by WS. Leaf RWC was similar between treatments under WW conditions, but interestingly it increased in T34 treatments (T34 and T34+Q) while it decreased in Q under WS (Fig. 3). These changes were similarly achieved for WUE, although they were not statistically significant. In the last growth stage, at 104 DAS, when the cob was ripening and the plant was starting to dry out, we found that An and g_s (Fig. 2) decreased compared to previous growth stages. We were surprised to find that An in T34 and T34+Q under WS was higher than in the Q treatment. However, g_s was similarly decreased by WS in the different treatments but not significantly (Suppl. Table S1). Associated with these changes under WS, we observed higher Fv'/Fm' and WUE in the T34 and T34+Q treatments compared to Q, with these values being even higher when only T34 was applied. Leaf

RWC was strongly decreased by WS compared to WW, while it was higher in T34 treatments, particularly in T34+Q (Suppl. Table S1).

Elemental nutrient composition and isotopic measurements

Leaf mineral nutrient content and C and N isotope compositions at 85 and 104 DAS (Table 2) showed that the water regime significantly affected nutritional components including N, C/N, K, P, S, Mn, B, and Zn, as well as $\delta^{15}N$. Particularly, WS decreased N, P, S, Mn, B and Zn in all the treatments at 85 DAS which continued decreased at 104 DAS but now also K was increased. The $\delta^{15}N$ increased under WS at both times, while the significant decrease in N led to an increase in the C/N ratio in leaves (Suppl. Table S2). Meanwhile, treatment and interaction effects were barely significant: S and Zn were significantly modified by treatment, increasing their content in leaves in T34+Q at 85 DAS, but this effect disappeared at 104 DAS (Suppl. Table S2). B was higher in T34+Q compared to the other treatments under WW at 104 DAS, while it was higher in T34 under WS (Suppl. Table S2). Results of kernel mineral nutrient content and C and N isotope compositions showed significant differences by treatment and water regime (Table 3, Suppl. Table S3 and S4). C and P contents were higher in T34 and T34+Q treatments than in Q treatment, being particularly higher in T34. WS conditions altered kernel nutrient and isotope composition (Table 3): N, P, Mg, Zn and $\delta^{13}C$ decreased by WS while Fe, C/N and $\delta^{15}N$ increased. The treatment x water regime interaction did not modify these parameters in the kernel, except for the $\delta^{13}C$ (Suppl. Table S3 and 4). It was strongly affected by WS in Q treatment and in a lesser extent in T34+Q, being not affected in T34.

Yield parameters

WS did not affect plant height but decreased shoot fresh/dry weight and dry weight of kernels per plant (Fig. 4, Suppl. Table S5). Shoot fresh weight was higher in T34 and T34+Q compared to Q, while a similar tendency was observed for shoot dry weight although it did not reach statistical significance. T34 treatment showed the highest number of kernels per plant compared to Q and T34+Q (Fig. 4), while the effects of water regime or the interaction were not significant (Suppl. Table S5). Dry weight of kernels per plant was significantly higher in T34 and T34+Q compared to Q under WW, while a similar non-significant tendency was observed under WS.

Discussion

246 The seed coating applied in this assay with *T. asperellum*, strain T34 was effective and the presence of the
247 chemical fungicide did not affect the initial populations of maize plants (Fig. 1; Table 1). In general, many
248 previous studies (Lifshitz 1986; Harman 1991; Prasad et al. 2002; Mastouri et al. 2010) showed that seed
249 coatings excipients used in scientific publications (talc, pelgel, gum arabic, corn starch, methyl cellulose,
250 polyethylene glycol) differ from those used industrially in seed companies (synthetic microplastic-based
251 polymers like the used here) and therefore are not comparable with the seed treatment used in our work.
252 Chemical treatment alone presented low T34 populations due to initial cross-contamination, but it was
253 insignificant compared to the treated seeds. Nevertheless, those populations in the rhizosphere remained in
254 a similar range to naturally occurring populations of *Trichoderma* spp. in soil: 10^2-10^3 CFU·g^{-1} dry weight
255 of soil (Longa et al. 2009) and were not altered by water regime (Table 1). At the end of the assay, the
256 rhizosphere populations were significantly much higher in the seed treatments with T34 than in T34+Q,
257 demonstrating the capacity of T34 to survive and establish itself in an inert soil from only an impregnated
258 seed. As expected, the presence of the chemical fungicide lowered its populations. Interestingly, the
259 interaction showed that populations increased under WS in T34+Q, but decreased in T34 in comparison to
260 their respective WW treatment. This could mean that the plant may modulate T34 populations as needed,
261 suggesting also that 10^4 cfu·g^{-1} may be inadequate for WS and 10^5 cfu·g^{-1} may be preferable. Other
262 experiments with field trials showed that T34 can be found at 10^4 CFU·g^{-1} rhizosphere dry weight at the
263 beginning of the maize reproductive stage (Fitó seeds, unpublished data) with the same maize hybrid, seed
264 coating (T34+Q) and in optimal conditions. In Pandey et al. (2016), dose-dependent *T. harzianum* strain
265 Th-56 showed a differentiated water-stress response in different rice genotypes, suggesting the need for a
266 minimal dose of *T. harzianum* to influence crop physiology under drought, which correlates with the effects
267 observed in our experiment.

268 In our experiment, water and treatment effects were more evident in late stages (grain filling) when drought
269 was more evident and can strongly decrease kernel yield, in agreement with previous reports (Anami et al.
270 2009; Bista et al. 2018). However, in the case of photosynthetic capacity, An was significantly modified in
271 early (63DAS) and late (104 DAS) stages, but not at 85 DAS, which could be associated with the specific
272 genotype used in this experiment. In this sense, the fact that Fv'/Fm' and g_s were mainly affected in late
273 growth stages when water supply was very limited demonstrated that the photosynthetic machinery of our
274 maize hybrid showed a certain drought tolerance. WS only decreased the C_i/C_a ratio and leaf RWC
275 compared to WW at 63 DAS, changes that did not modify photosynthetic capacity. At 85 DAS, An

276 decreased slightly under WS together with E, mainly due to stomatal closure (lower g_s) and a lower
277 maximum quantum efficiency of photosystem II (Fv'/Fm'; Fig. 1, Suppl. Table S1). When water
278 deprivation was intensified, coinciding with grain filling stage (104 DAS), we observed the same changes
279 on photosynthetic capacity as before but more severe, also explained by a decrease in leaf water status
280 (WUE, RWC) and CO_2 available for its fixation by Rubisco (C_i/C_a ratio). It is known that An decreases
281 when water is scarce, due mainly to stomatal closure or inhibition of CO_2 metabolism (Asada et al. 2000;
282 Javed et al. 2011; Vitale et al. 2011; Aslam et al. 2013), which is in agreement with our results. $\delta^{13}C$ values
283 are known to decrease with increasing water stress in maize and other C_4 plants (Dercon et al. 2006). In our
284 study, kernel $\delta^{13}C$ decreased under WS (Table 3 and Suppl. Table S3), highlighting that the plants suffered
285 water stress during the experiment regardless of the treatment.

286 Regarding the treatment effects, at early stages (63 DAS) T34 treatments (T34 and T34+Q) promoted
287 photosynthesis and transpiration compared to Q, particularly under WS (Fig. 1, Suppl. Table S1). Anyhow,
288 T34 treatments showed some features suggesting a better water status, as an increase in E in T34+Q under
289 WW or leaf RWC in T34 and T34+Q compared to Q. During the key growth stage of grain filling (104
290 DAS), and especially when water was severely limited in WS conditions, mimicking real conditions for
291 field-grown maize in the Mediterranean basin, greater differences were observed. Although T34 seemed to
292 not have an effect on photosynthetic capacity when water supply was optimal, it really influenced it under
293 WS conditions. Indeed, application of T34 (T34 and T34+Q treatments) increased An and E by an
294 improvement of light harvesting (Fv'/Fm') and leaf water status (WUE and RWC). These changes can also
295 suggest the absence or reduction of damage to the photosynthetic machinery caused by drought stress.
296 Similar improvements in quantum efficiency were observed with *T. harzianum* T22 in *Arabidopsis*
297 (Shoresh et al. 2010). Moreover, kernel $\delta^{13}C$ results showed higher values in the T34 and T34+Q treatments
298 than in Q under WS (Suppl. Table S3), which may indicate that T34 improved water status throughout the
299 experiment, since $\delta^{13}C$ provides information on the photosynthetic carbon assimilation and is a time-
300 integrated indicator of the A_n/g_s ratio and WUE (Serret et al. 2018). The increase in shoot fresh weight in
301 T34 and T34+Q compared to Q, but not significantly in shoot dry weight, clearly indicated that water
302 content at whole plant level was promoted by T34 application. In carnation plants, enhancement of water
303 uptake related to T34 is known, even in the presence of biotic pressure (Sant et al. 2010).

Leaf mineral nutrient content was modified by WS, indicating that the uptake of several nutrients (i.e., N, P, S, Mn, B and Zn) decreased due to this stress, except in the case of K at 104 DAS (Table 2 and Suppl. Table S2). $\delta^{15}N$ was modified by water conditions in leaves at two growth stages and in kernels at harvest. The nitrogen isotope composition ($\delta^{15}N$) is used to trace shifts in nitrogen metabolism, although it could be related to several processes and the underlying biochemical mechanisms is still not very well understood (Medina et al. 2016; Serret et al. 2018). In our experiment, its changes highlighted that it is a good indicator of changes in N metabolism, since the N content was affected significantly in leaves and kernels (Tables 2 and 3). Drought decreases nutrient uptake in most plants for several reasons, including a reduction of nutrient supply through mineralization and reduced nutrient diffusion in the soil (Bista et al. 2018). Related with this, E and g_s decreased in our experiment, which could directly influence nutrient uptake. These changes affected final kernel quality, decreasing the content of nutrients such as N, P and Zn, although Fe increased (Table 3). In many cases, drought stress lead to Fe deficiencies in plant (Ahanger et al. 2016). However, in our case its higher kernel content could reflect a greater Fe uptake and/or allocation during this experiment and can be related with the lower content of P and Zn, which are known to have opposite effects on Fe uptake (Ahanger et al. 2016).

The effects of T34 application on mineral nutrient content in leaves was scare. Although some nutrients (S and Zn) increased in T34+Q leaves compared to the other treatments at 85 DAS, these changes disappeared at 104 DAS. In the case of B, it increased in leaves of T34+Q under WW and T34 under WS. Its uptake is mostly a passive process so it is greatly determined by the rate of water uptake by roots (Ahanger et al. 2016). Its higher content in leaves, at least in T34 under WS, could be related with the improvement of photosynthesis by the stabilization of membrane integrity and reduction of ROS (Ahanger et al. 2016), although further studies are needed to corroborate this hypothesis. C and P contents were higher in kernels in T34 and T34+Q compared to Q (Table 3), suggesting higher C assimilation, P uptake and/or translocation to kernels. Similarly, García-López et al. (2015) showed that plants treated with T34 improved P absorption in the presence of non-available P forms in cucumber. In Fernández et al. (2014), improvements of nutrient assimilation and C allocation, as well as other growth parameters were reported in T34-treated tomato plants. In spite of this better C content in kernels and photosynthetic machinery and leaf water status under WS, T34 did not prevent a decrease in kernel weight, suggesting that the drought stress performed in this study was severe. The maize responses to T34 under WS reported here demonstrate that the T34 population levels we measured are adequate to prevent water loss and promote photosynthetic capacity and stress

avoidance in maize leaves but not sufficient to improve kernel dry weight and, then, grain yield under WS conditions. Nevertheless, T34 did promote kernel yield when the water regime was more favorable (i.e. WW conditions), highlighting the potential of T34 to improve maize yield under future climate change scenario and/or to reduce environmental contamination. Speculating about the mechanism by which T34 could alleviate drought stress in our experiment, authors such as Alwhibi et al. (2017) observed that *T. harzianum* increased phenol and flavonoid content in tomato plants affected by drought. Meanwhile, *T. asperelloides* T203 ameliorated plant growth under biotic stress by lowering deleterious ethylene (that accumulates under stress) thanks to increased antioxidant activity (Shoresh and Harman 2008; Brotman et al. 2013) which is again related to higher phenol synthesis (Ahmad et al. 2017). Increased antioxidant levels would degrade higher amounts of ROS (reactive oxygen species) and therefore protect photosynthesis. Harman et al. (2004) suggested that *Trichoderma* spp. metabolites or root colonization could modulate cellular and molecular changes in plants through modification of plant gene expression, thus causing a shift in plant responses.

In conclusion, in this work we observe the potential of T34 seed treatment under optimal conditions and abiotic stress in a key crop such as maize, which is susceptible to water stress around the world. T34 enhanced hydration and photosynthetic capacity under WS conditions. However, it was not sufficient to avert kernel dry weight decrease, probably due to the severity of the stress. In the context of climate change in Mediterranean regions, where periods of drought are increasingly present, the use of biological plant protection products against soil diseases (such as T34) can have secondary benefits for crops either under optimal conditions or under drought stress. Further studies are necessary to evaluate the positive effects of T34 application on leaf water status and photosynthetic capacity under different water regimes from mild to severe water stress, to understand its effects on plant metabolism and yield improvements, particularly in field-grown conditions and using a wider spectrum of maize genotypes with differentiated drought sensibility.

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Author contributions

VE performed the assays, collected kernels, sampled and analyzed tissues, analyzed the data and wrote the manuscript. RV and OV performed the gas exchange measurements and contributed to the writing and critical revision of the manuscript. JJN helped design and perform the seed treatments and critically reviewed the manuscript. MIT provided technical advice concerning T34 application, experimental design and analysis of the results, and reviewed the manuscript.

Abbreviations

WW: well-watered conditions/plants

WS: water-stressed conditions/plants

RWC: relative water content

An: rate of photosynthesis

E: transpiration

Fv'/Fm': maximum quantum efficiency of photosystem II (PSII)

$\delta^{13}C$: stable carbon isotopic composition

$\delta^{15}N$: stable nitrogen isotopic composition

Bibliography

Ahanger MA, Morad-Talab N, Abd-Allah EF, Ahmad P, Hajiboland R (2016) Plant growth under drought stress. In: Ahmad P (ed) Water Stress and Crop Plants, Wiley, New Jersey, pp 649-668. https://doi.org/10.1002/9781119054450.ch37

Ahmad I, Basra SMA, Akram M., Wasaya A, Ansar M, Hussain S (2017) Improvement of antioxidant activities and yield of spring maize through seed priming and foliar application of plant growth regulators under heat stress conditions. Semina:Ciencias Agrarias 38:47-56.

https://doi.org/10.5433/1679-0359.2017v38n1p47

Alwhibi MS, Hashem A, AbdAllah EF, Alqarawi AA, Soliman DWK., Wirth S, Egamberdieva D (2017) Increased resistance of drought by Trichoderma harzianum fungal treatment correlates with increased secondary metabolites and proline content. J Integr Agric 16:1751–1757. https://doi.org/10.1016/S2095-3119(17)61695-2

Anami S, De Block M, MacHuka J, Van Lijsebettens M (2009) Molecular improvement of tropical maize for drought stress tolerance in Sub-Saharan Africa. Crit Rev Plant Sci 28:16–35. https://doi.org/10.1080/07352680802665305

Asada K, Allen J, Foyer CH, Matthijs, HCP (2000) The water-water cycle as alternative photon and electron sinks. Philos Trans R Soc Lond B Biol Sci 355:1419–1431. https://doi.org/10.1098/rstb.2000.0703

Aslam M, Zamir I, Afzal I, Yaseen M, Mubeen M, Shoaib A (2013) Drought tolerance in maize through Potassium: Drought stress, its effect on maize production and development of drought tolerance through potassium application. Agronomic Research in Moldavia 46:99-144.

Barrs HD, Weatherley PE (1962) A Re-Examination of the Relative Turgidity Technique for Estimating Water Deficits in Leaves. Aust J Biol Sci 15:413-428. https://doi.org/10.1071/BI9620413

Bista DR, Heckathorn SA, Jayawardena DM, Mishra S, Boldt JK (2018) Effects of drought on nutrient uptake and the levels of nutrient-uptake proteins in roots of drought-sensitive and -tolerant grasses. Plants 7:28. https://doi.org/10.3390/plants7020028

Borrero C, Trillas MI, Delgado A, Avilés M (2012) Effect of ammonium/nitrate ratio in nutrient solution on control of Fusarium wilt of tomato by Trichoderma asperellum T34. Plant Pathol 61:132–139. https://doi.org/10.1111/j.1365-3059.2011.02490.x

Brotman Y, Landau U, Cuadros-Inostroza Á, Takayuki T, Fernie AR, Chet I, Viterbo A, Willmitzer L (2013) Trichoderma-Plant Root Colonization: Escaping Early Plant Defense Responses and Activation of the Antioxidant Machinery for Saline Stress Tolerance. PLoS Pathog 9:e1003221 https://doi.org/10.1371/annotation/8b818c15-3fe0-4e56-9be2-e44fd1ed3fae

Chung YR, Hoitink HAJ (1990) Interactions between Thermophilic Fungi and Trichoderma hamatum in

415 suppression of Rhizoctonia Damping-off in a Bark Compost-Amended Container Medium.
416 Phytopathology 80:73–77.

417 Cotxarrera L, Trillas-Gay MI, Steinberg C, Alabouvette C (2002) Use of sewage sludge compost and
418 Trichoderma asperullum isolates to suppress fusarium wilt of tomato. Soil Biol Biochem 34:467–
419 476. https://doi.org/10.1016/S0038-0717(01)00205-X

420 de Santiago A, García-López AM, Quintero JM, Avilés M, Delgado A (2013) Effect of Trichoderma
421 asperellum strain T34 and glucose addition on iron nutrition in cucumber grown on calcareous
422 soils. Soil Biol Biochem 57:598–605. https://doi.org/10.1016/j.soilbio.2012.06.020

423 de Santiago A, Quintero JM, Avilés M, Delgado A (2011). Effect of Trichoderma asperellum strain T34
424 on iron, copper, manganese, and zinc uptake by wheat grown on a calcareous medium. Plant Soil
425 342:97–104. https://doi.org/10.1007/s11104-010-0670-1

426 Dercon G, Clymans E, Diels J, Merckx R, Deckers J (2006) Differential δ13C isotopic discrimination in
427 maize at varying water stress and at low to high nitrogen availability. Plant Soil 282:313–326.
428 https://doi.org/10.1007/s11104-006-0001-8

429 Farooq M, Wahid A, Kobayashi N, Fujita D, Basra SMA (2009) Plant drought stress: effects,
430 mechanisms and management. Agron Sustain Dev 29:185–212.
431 https://doi.org/10.1051/agro:2008021

432 Fernández E, Segarra G, Trillas MI (2014) Physiological effects of the induction of resistance by compost
433 or Trichoderma asperellum strain T34 against Botrytis cinerea in tomato. Biol Control 78:77–85.
434 https://doi.org/10.1016/j.biocontrol.2014.06.012

435 Fu J, Liu Z, Li Z, Wang Y,Yang K (2017) Alleviation of the effects of saline-alkaline stress on maize
436 seedlings by regulation of active oxygen metabolism by Trichoderma asperellum. PLoS One
437 12:e0179617. https://doi.org/10.1371/journal.pone.0179617

438 García-López AM, Avilés M, Delgado A (2015) Plant uptake of phosphorus from sparingly available P-
439 sources as affected by Trichoderma asperellum T34. Agr Food Sci 24:249–260.
440 https://doi.org/10.23986/afsci.49532

441 Gholami A, Shahsavani S, Nezarat S (2009) The effect of plant growth promoting rhizobacteria (PGPR)

442 on germination, seedling growth and yield of maize. Int J Agr Biol Eng 1:35–40.

443 Hanway JJ, Ritchie SW (1986) How a corn plant develops. Special Report 48, Iowa State University
444 Extension, Ames.

445 Harman GE (1991) Seed treatments for biological control of plant disease. Crop Prot 10:166–171.
446 https://doi.org/10.1016/0261-2194(91)90038-S

447 Harman GE, Howell CR, Viterbo A, Chet I, Lorito M (2004) Trichoderma species - Opportunistic,
448 avirulent plant symbionts. Nat Rev Microbiol 2:43–56. https://doi.org/10.1038/nrmicro797

449 Howell CR (2003) Mechanisms Employed by Trichoderma Species in the Biological Control of Plant
450 Diseases: The History and Evolution of Current Concepts. Plant Dis 87:4-10.
451 https://doi.org/10.1094/PDIS.2003.87.1.4

452 Hu X, Roberts DP, Maul JE, Emche SE, Liao X, Guo X, Liu S (2011) Formulations of the endophytic
453 bacterium Bacillus subtilis Tu-100 suppress Sclerotinia sclerotiorum on oilseed rape and improve
454 plant vigor in field trials conducted at separate locations. Can J Microbiol 57:539–546.
455 https://doi.org/10.1139/w11-041

456 Javed N, Ashraf M, Akram NA, Al-Qurainy F (2011) Alleviation of adverse effects of drought stress on
457 growth and some potential physiological attributes in maize (Zea mays L.) by seed electromagnetic
458 treatment. Photochem Photobiol 87:1354–1362. https://doi.org/10.1111/j.1751-1097.2011.00990.x

459 Krzyzinska B, Glazek M, Maczynska A (2005) Seed treatment for control leaf spot diseases of spring
460 wheat. Acta Agrobotanica 58:37–43. https://doi.org/10.5586/aa.2005.006

461 Lifshitz R, Windham M, Baker R (1986) Mechanism of Biological Control of Preemergence Damping-off
462 of Pea by Seed Treatment with Trichoderma spp. Phytopathology 76:720- 725.

463 Longa CMO, Savazzini F, Tosi S, Elad Y, Pertot I (2009) Evaluating the survival and environmental fate
464 of the biocontrol agent trichoderma atroviride SC1 in vineyards in northern Italy. J Appl Microbiol
465 106:1549–1557. https://doi.org/10.1111/j.1365-2672.2008.04117.x

466 Lorito M, Woo SL, Harman GE, Monte E (2010) Translational Research on Trichoderma: From 'Omics
467 to the Field. Ann. Rev. Phytopathol 48:395–417. https://doi.org/10.1146/annurev-phyto-073009-
468 114314

469 Mastouri F, Björkman T, Harman GE (2010) Seed Treatment with Trichoderma harzianum Alleviates
470 Biotic, Abiotic, and Physiological Stresses in Germinating Seeds and Seedlings. Phytopathology
471 100:1213–1221. https://doi.org/10.1094/phyto-03-10-0091

472 Medina S, Vicente R, Amador A, Araus JL (2016) Interactive Effects of Elevated [CO2] and Water Stress
473 on Physiological Traits and Gene Expression during Vegetative Growth in Four Durum Wheat
474 Genotypes. Front Plant Sci 7:1738. https://doi.org/10.3389/fpls.2016.01738

475 Moya-Elizondo EA, Jacobsen, BJ (2016) Integrated management of Fusarium crown rot of wheat using
476 fungicide seed treatment, cultivar resistance, and induction of systemic acquired resistance
477 (SAR). Biol Control 92:153–163. https://doi.org/10.1016/j.biocontrol.2015.10.006

478 Ortiz N, Armada E, Duque E, Roldán A, Azcón R (2015) Contribution of arbuscular mycorrhizal fungi
479 and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of
480 autochthonous or allochthonous strains. J Plant Physiol 174:87–96.
481 https://doi.org/10.1016/j.jplph.2014.08.019

482 Pandey V, Ansari MW, Tula S, Yadav S, Sahoo RK, Shukla N, Tuteja N (2016) Dose-dependent
483 response of Trichoderma harzianum in improving drought tolerance in rice genotypes. Planta
484 243:1251–1264. https://doi.org/10.1007/s00425-016-2482-x

485 Prasad RD, Rangeshwaran R, Hegde SV, Anuroop CP (2002) Effect of soil and seed application of
486 Trichoderma harzianum on pigeonpea wilt caused by Fusarium udum under field conditions. Crop
487 Prot 21:293–297. https://doi.org/10.1016/S0261-2194(01)00100-4

488 Sant D, Casanova E, Segarra G, Avilés M, Reis M, Trillas MI (2010) Effect of Trichoderma asperellum
489 strain T34 on Fusarium wilt and water usage in carnation grown on compost-based growth medium.
490 Biol Control 53:291–296. https://doi.org/10.1016/j.biocontrol.2010.01.012

491 Segarra G, Aviles M, Casanova E, Borrero C, Trillas I (2013) Effectiveness of biological control of
492 Phytophthora capsici in pepper by Trichoderma asperellum strain T34. Phytopathologia
493 Mediterranea 52:77–83. https://doi.org/10.14601/Phytopathol_Mediterr-11242

494 Segarra G, Casanova E, Avilés M, Trillas I (2010) Trichoderma asperellum strain T34 controls Fusarium
495 wilt disease in tomato plants in soilless culture through competition for iron. Microb Ecol 59:141–

149. https://doi.org/10.1007/s00248-009-9545-5

Segarra G, Casanova E, Bellido D, Odena MA, Oliveira E, Trillas I (2007) Proteome, salicylic acid, and jasmonic acid changes in cucumber plants inoculated with Trichoderma asperellum strain T34. Proteomics 7:3943–3952. https://doi.org/10.1002/pmic.200700173

Serret MD, Yousfi S, Vicente R, Piñero MC, Otálora-Alcón G, del Amor FM, Araus JL (2018) Interactive Effects of CO2 Concentration and Water Regime on Stable Isotope Signatures, Nitrogen Assimilation and Growth in Sweet Pepper. Front Plant Sci 8:2180. https://doi.org/10.3389/fpls.2017.02180

Shoresh M, Harman GE (2008) The molecular basis of shoot responses of maize seedlings to Trichoderma harzianum T22 inoculation of the root: A proteomic approach. Plant Physiol 147:2147–2163. https://doi.org/10.1104/pp.108.123810

Shoresh M, Harman GE, Mastouri F (2010) Induced Systemic Resistance and Plant Responses to Fungal Biocontrol Agents. Annu. Rev. Phytopathol 48:21–43. https://doi.org/10.1146/annurev-phyto-073009-114450

Singh A, Parmar N, Kuhad RC, Ward OP (2011) Bioaugmentation, Biostimulation and Biocontrol. Springer, Heidelberg

Taylor AG, Min TG, Harman GE, Jin X (1991) Liquid coating formulation for the application of biological seed treatments of Trichoderma harzianum. Biol Control 1:16–22. https://doi.org/10.1016/1049-9644(91)90096-I

Trillas MI, Casanova E, Cotxarrera L, Ordovás J, Borrero C, Avilés M (2006) Composts from agricultural waste and the Trichoderma asperellum strain T-34 suppress Rhizoctonia solani in cucumber seedlings. Biol Control 39:32–38. https://doi.org/10.1016/j.biocontrol.2006.05.007

Verma M, Brar, SK, Tyagi, RD, Surampalli RY, Valéro JR (2007) Antagonistic fungi, Trichoderma spp: Panoply of biological control. Biochem Eng J 37:1–20. https://doi.org/10.1016/j.bej.2007.05.012

Vitale L, Arena C, Carillo P, di Tommasi P, Mesolella B, Nacca F, Magliulo V (2011) Gas exchange and leaf metabolism of irrigated maize at different growth stages. Plant Biosyst 145:485–494. https://doi.org/10.1080/11263504.2011.562373

Zeun R, Scalliet G, Oostendorp M (2013) Biological activity of sedaxane - a novel broad-spectrum fungicide for seed treatment. Pest Manag Sci 69:527–534. https://doi.org/10.1002/ps.3405

Figures

Fig. 1 Scanning electron microscopy (SEM) images of corn seed treated with *T. asperellum* strain T34. a) and b): Conidia adhered to the surface of a seed corn just after T34 treatment. c) and d): Germinated hyphae and/or conidia of T34 on germinated corn seed.

Fig. 2 Gas exchange and fluorescence measurements. Rate of photosynthesis (*An, µmol* $CO_2 \cdot m^{-2} \cdot s^{-1}$), transpiration (*E, nmol* $H_20 \cdot m^{-2} \cdot s^{-1}$) and maximum efficiency of photosystem II (*Fv'/Fm'*) were recorded in well-watered (WW) and water-stressed (WS) plants at three time points (63, 85 and 104 days after sowing: DAS) and for three different seed treatments (*T. asperellum* strain T34 (T34); chemical fungicide (Q); and their combination (T34+Q)). Plants were subjected to mild stress for 28 days (until 63 DAS), and intensified stress for the rest of the assay (until 85 and 104 DAS). Each value is the mean of 3-5 replicates per treatment and condition. Significant effects were determined by two-way ANOVA ($P < 0.05$). Different letters denote statistical significance for the interaction. Figures with no letters showed no significant differences in the interaction. Mean values in Suppl. Table S1.

Fig. 3 Water use efficiency (*WUE*) and leaf relative water content (*RWC*) were recorded in well-watered (WW) and water-stressed (WS) plants at three time points (63, 85 and 104 days after sowing: DAS) with three different seed treatments (*T. asperellum* strain T34 (T34); chemical fungicide (Q) and their combination (T34+Q)). Plants were subjected to mild stress for 28 days (until 63 DAS), and intensified stress for the rest of the assay (until 85 and 104 DAS). Each value is the mean of 3-5 replicates (n = 3-5). Significant effects were determined by two-way ANOVA ($P < 0.05$). Different letters denote statistical significance for the interaction. Figures with no letters showed no significant differences in the interaction. Mean values in Suppl. Table S1.

Fig. 4 Kernel parameters: dry weight of kernels (a) and number of kernels (b) at the end of the greenhouse study (113 days after sowing: DAS) of an experimental maize (*Zea mays*) variety grown under different seed treatments (*T. asperellum* strain T34 (T34); chemical fungicide (Q); and their combination (T34+Q)). The plants were subjected to mild stress for 28 days (until 63 DAS), and intensified stress for the rest of the assay (until 85 and 104 DAS). Each value is the mean of 4-7 replicates. Significant effects were determined

by two-way ANOVA ($P < 0.05$). Different letters denote statistical significance for the interaction. Mean values in Suppl. Table S5.

Table 1 T34 population in the seeds at the beginning of the experiment and in the rhizosphere at 104 days after sowing. T34: *T. asperellum* T34 seed treatment; Q: chemical fungicide seed treatment; T34+Q: combination of *T. asperellum* T34 and chemical fungicide; WW: well-watered, WS: water-stressed (WS). CFU: colony forming units of T34 per gram of dry weight of substrate.

Beginning of assay (treated seeds)[a]				
Log (CFU of T34·seed^{-1})				
T34	6.08 ± 0.07 b		P-value	LSD value
Q	1.09 ± 0.11 a	Log (CFU of T34·kernel^{-1})		
T34+Q	6.31 ± 0.03 b	T	<0.001	0.2497
End of assay (rhizosphere)[b]				
Log (CFU of T34·(g of dry weight of substrate)$^{-1}$)				
T34	5.22 ± 0.03 c		P-value	LSD value
Q	2.14 ± 0.03 a	Log (CFU of T34·(g of dry weight of substrate)$^{-1}$)		
T34+Q	4.30 ± 0.05 b	T	<0.001	0.0981
WW	3.87 ± 0.22 a			
WS	3.90 ± 0.22 a	C	0.461	0.0801
T34 WW	5.33 ± 0.03 e			
T34 WS	5.10 ± 0.04 d			
Q WW	2.17 ± 0.03 a			
Q WS	2.10 ± 0.06 a			
T34+Q WW	4.11 ± 0.06 b			
T34+Q WS	4.50 ± 0.03 c	T*C	<0.001	0.1387

[a,b] For the treated seed and rhizosphere populations, values were log-transformed mean values ± SE of 3-5 seeds or five pooled pots, respectively, for each treatment and condition, repeated twice. Significant effects were determined by two-way ANOVA ($P < 0.05$) plus Fisher's LSD. Different letters in each section indicate significant differences ($P < 0.05$).

Table 2 P-value of leaf concentration, stable isotopic composition (‰), macro- and micronutrients at 85 and 104 days after sowing (DAS). T: seed treatments (*T. asperellum* strain T34, chemical fungicide and their combination), C: water condition (well-watered and water-stressed), and TxC: interaction.

	85 DAS			104 DAS		
	T	C	TxC	T	C	TxC
C	0.562	0.739	0.308	0.424	0.086	0.221
$\delta^{13}C$	0.861	0.943	0.648	0.284	0.474	0.702
N	0.694	**0.027**	0.313	0.356	**<0.001**	0.543
$\delta^{15}N$	0.289	**<0.001**	0.446	0.445	**<0.001**	0.646
C/N	0.896	**0.007**	0.421	0.633	**<0.001**	0.637
K	0.657	0.45	0.424	0.563	**0.029**	0.286
Ca	0.105	0.107	0.826	0.218	0.113	0.643
Mg	0.873	0.086	0.598	0.371	0.131	0.09
P	0.176	**<0.001**	0.668	0.681	**<0.001**	0.144
S	**0.031**	**<0.001**	0.257	0.817	**<0.001**	0.286
Fe	0.719	0.369	0.178	0.765	0.095	0.102
Mn	0.556	**<0.001**	0.163	0.416	**<0.001**	0.658
Cu	0.724	0.056	0.798	0.288	0.303	0.141
B	0.602	**<0.001**	0.533	0.5	**<0.001**	**0.01**
Zn	**<0.001**	**<0.001**	**0.013**	0.546	**0.018**	0.974

Each value is the mean of 3 replicates. Significant effects were determined by two-way ANOVA. Different letters denote a statistical significance in Fisher's LSD *post hoc* ($P < 0.05$). C, N, K, Ca, Mg, P, S, Na and Fe: mg of nutrient· (g leaf dry weight)$^{-1}$. Mn, Cu, B and Zn: µg of nutrient·(g leaf dry weight)$^{-1}$. Mean values can be found in Suppl. Table S2.

Table 3 C and N content and stable isotopic composition (‰), C/N ratio, and content of other macro- and micronutrients in kernels at the end of the assay (113 days). Measurements were taken in well-watered (WW) and water-stressed (WS) plants, with the following seed treatments: *T. asperellum* strain T34 (T34), chemical fungicide (Q) and their combination (T34+Q).

	C	$\delta^{13}C$	N	$\delta^{15}N$	C/N
T34	**425.14 ± 2.58 b**	-11.799 ± 0.04 a	15.83 ± 0.39 a	2.06 ± 0.45 a	26.98 ± 0.60 a
Q	**416.04 ± 2.24 a**	-11.883 ± 0.09 a	15.70 ± 0.44 a	1.79 ± 0.50 a	26.67 ± 0.69 a
T34+Q	**422.03 ± 1.93 ab**	-11.757 ± 0.06 a	16.39 ± 0.50 a	2.14 ± 0.44 a	25.95 ± 0.73 a
WW	420.86 ± 1.82 a	**-11.662 ± 0.03 b**	**16.48 ± 0.39 b**	**0.69 ± 0.08 a**	**25.72 ± 0.57 a**
WS	421.28 ± 2.30 a	**-11.964 ± 0.04 a**	**15.46 ± 0.27 a**	**3.30 ± 0.17 b**	**27.35 ± 0.44 b**

	K	Ca	Mg	P	S	Fe	Zn
T34	0.96 ± 0.08 a	5.84 ± 0.44 a	0.31 ± 0.02 a	**0.85 ± 0.05 b**	0.24 ± 0.01 a	5.24 ± 0.53 a	5.05 ± 0.33 a
Q	0.84 ± 0.04 a	6.25 ± 0.26 a	0.33 ± 0.01 a	**0.72 ± 0.03 a**	0.28 ± 0.01 a	5.88 ± 0.59 a	6.01 ± 0.60 a
T34+Q	0.89 ± 0.07 a	5.61 ± 0.43 a	0.30 ± 0.02 a	**0.80 ± 0.02 ab**	0.28 ± 0.02 a	6.12 ± 0.85 a	5.85 ± 0.48 a
WW	0.92 ± 0.06 a	6.31 ± 0.29 a	**0.34 ± 0.01 b**	**0.85 ± 0.03 b**	0.28 ± 0.01 a	**4.48 ± 0.34 a**	**6.25 ± 0.39 b**
WS	0.87 ± 0.05 a	5.49 ± 0.28 a	**0.29 ± 0.01 a**	**0.73 ± 0.03 a**	0.25 ± 0.01 a	**7.01 ± 0.29 b**	**5.03 ± 0.30 a**

Each value is the mean of 3 replicates. Significant effects were determined by two-way ANOVA. Different letters denote a statistical significance in Fisher's LSD *post hoc* ($P < 0.05$). C, N, K, Mg, P and S (mg of nutrient) and Ca, Fe and Zn (µg of nutrient· (g kernel dry weight)$^{-1}$). Mean values of the interaction and p-values in Suppl. Tables S3 and S4.